COMPARATIVE STUDIES ON THE FLAVOUR COMPOUNDS OF MILK PRODUCED WITH UREA AND NORMAL FEEDING

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During the last ten years much work has been performed in studies on the flavour compounds of milk and milk products, and several chemical compounds have been identified. However, only incomplete information is available on the origin of these compounds. Theoretically, milk flavour substances can be classified into two groups: those flavour compounds which are transferred from fodder to milk *via* the organism and those which are formed in the organism (in the rumen and/or in the metabolic processes in the liver or mammary gland) from carbohydrates, amino- or fatty acids and other chemical compounds in the fodder.

It is a well known fact that certain fodder plants and silages, for example some cruciferous plants and some *Allium* species, can give rise to characteristic flavour defects in milk. Silage also can impart an extremely unpleasant off-flavour to milk if repulsive flavour substances have been formed in the silage by the effect of micro-organisms, as happens if the pH is not low enough. Also on feeding cows with various volatile organic compounds it is found that some of them may enter the milk *via* the digestive route[1,2] in such amounts that they can give distinct flavour defects to the milk.

With the object of eliminating the effect of the flavour compounds in normal fodder, studies on the production of milk with an odourless, purified, protein-free diet were begun in this laboratory in 1961. These studies, which later opened great possibilities for milk production with a high annual yield from cheap industrial raw materials, very soon showed that the taste and smell of the milk produced on purified feed and urea (named zero milk) were so close to normal that it was very difficult in organoleptic tests to differentiate between zero milk and normal milk. An accurate chemical analysis of the milk constituents was therefore necessary in order to establish differences or similarities between zero milk and normal milk. The major constituents of milk, especially protein and fat, have been investigated very thoroughly in this laboratory.[3]

The isolation of flavour compounds from milk for gas chromatographic analyses is difficult because the concentration of the flavour compounds is very low. Several methods such as solvent extraction, vacuum distillation, head space techniques and continuous molecular distillation of the milk fat have been used for this purpose. In the work reported here vacuum carbon dioxide distillation of the milk fat[4] was used for the isolation of flavour compounds of milk. Carbon dioxide distillation is simple in operation, the apparatus is inexpensive and the free fatty acid content of the flavour concentrate is considerably lower than that obtained by steam distillation.

In the studies on the effect of fodder on milk flavour only very small differences in general were detected, although the fodders used were quite different. Also, milk produced with urea-starch-cellulose-sucrose or urea-starch-cellulose-hemicellulose feeding contains the same main flavour compounds as those of normal milk. Typical gas chromatograms of milk samples produced with AIV silage-hay-meal-straw and urea-starch-cellulose-sucrose feeding are presented in Figs. 1 and 2 respectively. The peaks 1,2,3,4,7 and 8 have been identified as δ-C_6, δ-C_7, δ-C_8, δ-C_{10}, δ-C_{12} and δ-C_{14} lactones. In zero milk the total lactone content, especially the content of the odd carbon number δ-lactones, seems to be somewhat higher than in normal milk. Recently a new branched-chain δ-lactone compound (*trans*-4-methyl-5-hydroxy-hexanoic acid lactone) was isolated from zero milk. It is present in normal milk also, but its concentration in zero milk is about 20 times higher. The higher concentration of the odd carbon number δ-lactones and the branched-chain δ-lactone in zero milk is evidently connected with the increased fatty acid biosynthesis by rumen bacteria. It is known that the concentration of odd-carbon-number and branched-chain fatty acids in milk fat is increased when protein in feed is substituted by urea[3,5]. The formation of even-carbon-number δ-lactones is a normal function of the mammary gland (or liver) and the feeding has naturally no effect on it.

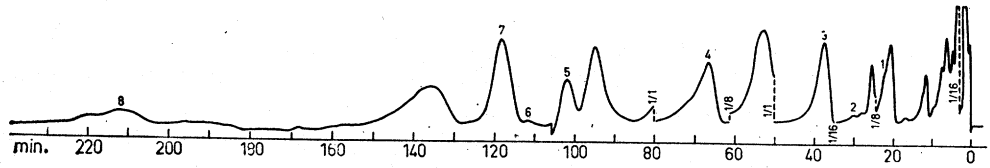


Fig. 1. Gas chromatogram of the flavour compounds of milk produced with AIV-silage-hay-meal-straw feeding.

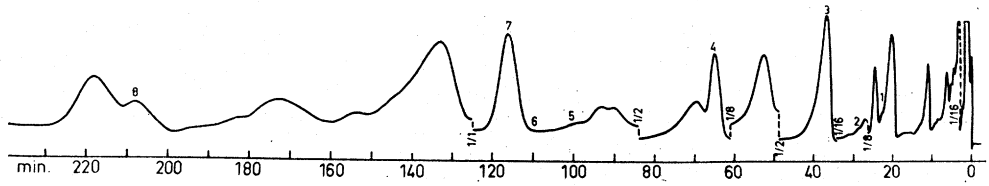
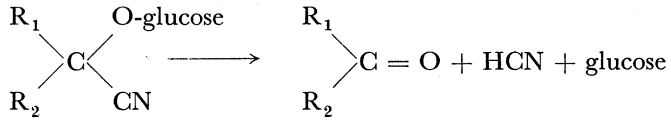


Fig. 2. Gas chromatogram of the flavour compounds of milk produced with urea-starch-cellulose-sucrose feeding.

The indole and skatole (peaks 5 and 6 in Figs. 1 and 2) contents in zero milk are considerably lower than in normal milk. Both of these compounds are normal metabolic degradation products of tryptophan. The lower concentration of these compounds in zero milk shows, however, that the compounds most likely partly originate from the fodder. Sometimes when cows are fed with mash or silage of bad quality (high indole and skatole contents) the concentration of indole and skatole in the milk rises to a still higher level and these compounds can then give detectable flavour defects.

Other aromatic compounds such as phenol, benzonitrile and benzothiazole have been identified in normal and zero milk, but all these compounds are probably artefacts. It is possible that the phenol originated from the air in the laboratory. The origin of benzonitrile is unknown. Scanlan *et al.*[6] detected benzonitrile in heated milk and assumed that this compound originates from cyanogenic glucosides in the fodder. We have found benzonitrile, however, in zero milk also. It is true that some cyanogenic glucosides occur in fodder plants (for example in some clover species), but in all cases hydrogen cyanide, a carbonyl compound and glucose are split off by enzymatic processes:



The benzothiazole found may have originated from rubber. 2-Mercaptobenzothiazole is an accelerator used in the manufacture of rubber and during the vulcanization process it loses sulphur, forming benzothiazole. No rubber components were used in the isolation procedures, but some of the components of the milking equipment were made of rubber. The concentration of all these compounds is, however, extremely low (only a few μg/kg milk fat) and evidently they have no effect on the flavour of milk.

Schwartz and Virtanen have found whole series of saturated aldehydes (from C_1 to C_{10}) and methylketones (from C_3 to C_{13}) in the volatile carbonyl fraction of normal and zero milk.[7] However, the 2-enals (from C_3 to C_{12}), which have been found in normal milk, are completely absent from zero milk.

As can be seen there are only very small differences between the flavour compounds in normal and zero milk. In general it can be said that the flavour of the 0-milk is always the same, being pleasant and without any disadvantageous off-flavours.

The only visible difference is the colour of the milk fat. Normal milk fat contains carotene, which is transferred from the grass to the milk and gives the typical yellow colour of milk fat. Because only vitamin A and no carotene is added to the test cows' diet the milk fat of zero milk is almost colourless. Carotene may have some effect on the taste of milk fat, but for the present its significance as a flavour compound is uncertain.

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